UNIVERSITÀ DEGLI STUDI DI PERUGIA

Department of Veterinary Medicine
MSc in Medical, Veterinary or Forensic Biotechnology

RISK ASSESSMENT FOR LISTERIA MONOCYTOGENES IN READY-TO-EAT FOODS

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INTRODUCTION TO RISK ANALYSIS

Food Safety represents a primary interest of the population and involves institutions, consumers, producers and the scientific world in a transversal way and with different roles

Scientific indications have become a reference standard for all countries of the EU, with the issuing of the Regulation (EC) no. 2073/2005 that has the goal of *ensuring safe food for the consumer* through the control of certain microbiological criteria

PROCESS HYGENE CRITERIA
(determines acceptability of production process)

FOOD SAFETY CRITERIA
(determines acceptability of a food product)

INTRODUCTION TO RISK ANALYSIS

Regulation (EC) No. 178/2002

- General principles and requirements of food law
- Gives direct responsibility to food business operators
- Establishes the European Food Safety Authority (E.F.S.A.)
- Introduction of the risk analysis tool

RISK ANALYSIS

- Describe the probability and potential impact of certain risks (**Risk Assessment**)
- Formulate decisions or propose alternatives to control them (**Risk Management**)
- Communicate to all stakeholders the results of the risk assessment and suggested corrective actions (**Risk Communication**)

RISK ASSESSMENT

- Science-based component of RA
- Aims to estimate the probability of harm resulting from human exposure to hazard present in foods
- Assist Risk Managers decisions
- Establishes scientifically-based food safety requirements between countries

Hazard Identification

HAZARD IDENTIFICATION

Hazard Identification

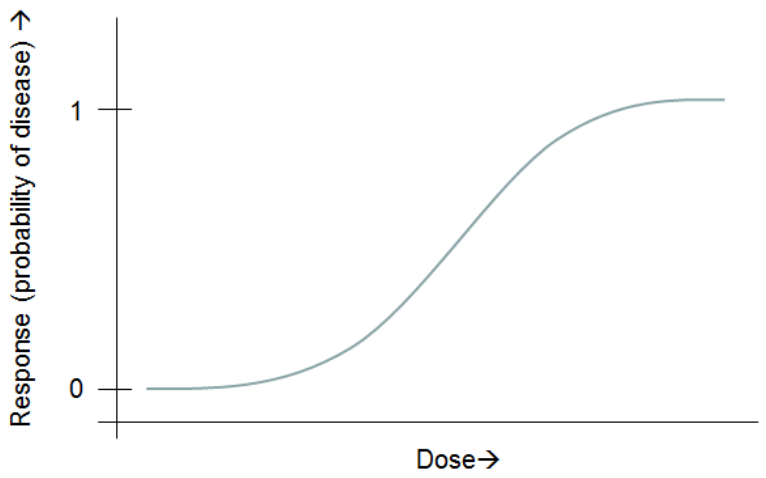
Qualitative process that involves the collection and evaluation of all available epidemiological data and all information on behavior of the hazard in the food, the effect of various factors and the pathogenesis. (mo, toxins, chemicals)

Hazard Characterisation

HAZARD CHARACTERIZATION

Hazard Characterisation

- Describes the nature, severity and duration of adverse health effect e.g. from ingestion of the microbial hazard. It also aims to develop a dose-response relationship between the amount of bacteria / toxin that people ingest and the likelihood that they will become ill
- Level of danger depend on the hazard type, on host sensitivity, on the type of the foo[illegible]ern



Exposure Assessment

EXPOSURE ASSESSMENT

Exposure Assessment

Determines the likelihood that an individual (or population) will be exposed to a hazard and the quantity likely to be ingested (e.g. CFU per serving)

The exposure assessment deals with the question of how the hazard is being introduced into the food chain and to what quantity the consumer will be exposed.

Influenced by the levels of microbial pathogens throughout the processing of food
(microbial ecology, initial contamination of the raw material, the processing methods applied, the packaging)

Generic Exposure Dose Equation

$$D = C \times IR \times AF \times EF / BW$$

D	=	exposure dose
C	=	contaminant concentration
IR	=	intake rate of contaminated medium
AF	=	bioavailability factor[1]
EF	=	exposure factor
BW	=	body weight.

consumption x contamination

Risk Characterisation

RISK CHARACTERISATION

Risk Characterisation

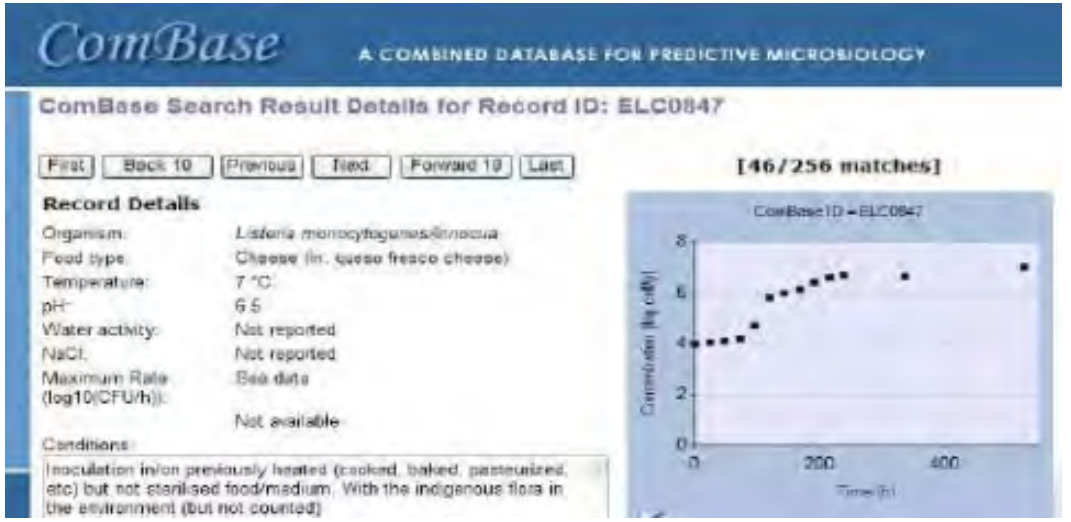
PROBABILITY	MAGNITUDE				
	Unimportant 1	Minor 2	Moderate 3	Major 4	Catastrophic 5
A (Assured)					
B (Expected)					
C (Possible)					
D (Improbable)					
E (Rare)					

Qualitative and/or quantitative estimation of the probability of occurrence and severity of known and potential adverse health effects with attendant uncertainties

Qualitative risk: low, medium, high
Quantitative risk: 20%, 100% or 1 up to 1000..

Hazard identification +
Hazard characterization +
Exposure assessment

Predictive microbiology



RISK MANAGEMENT

- Examination of the results of the risk assessment
- Development of policy, legislation, standards, guidelines, recommendations to reduce or prevent risks
- Influenced by economics, politics, legal context and societal perspectives
- Can commission risk assessments it self and often also leads on risk communication.

RISK COMMUNICATION

- Interactive exchange of information and opinions in the risk analysis process as regards hazards and risks, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, feed and food businesses, the academic community and other interested parties

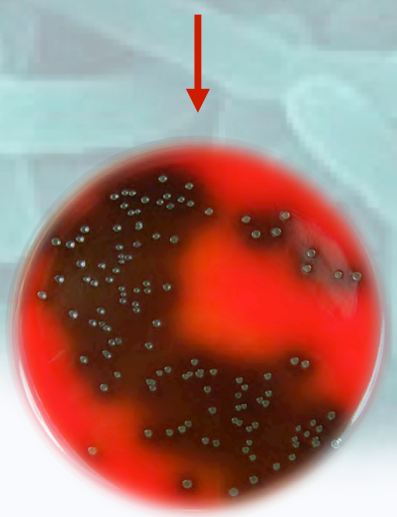
- Explanation of risk assessment findings and the basis of risk management decisions.

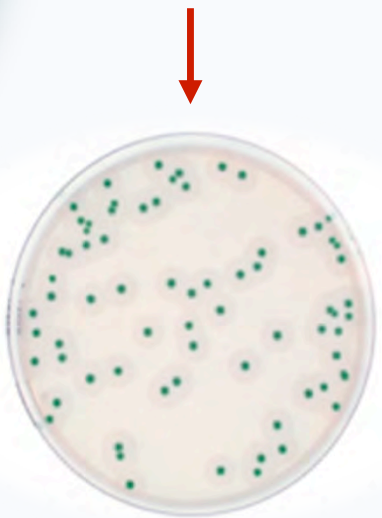
- Clearly communicate not only with key partners and stakeholders, but also with the general public, to help bridge the gap between science and consumer

1. HAZARD IDENTIFICATION

LISTERIA.. WHAT IS IT?

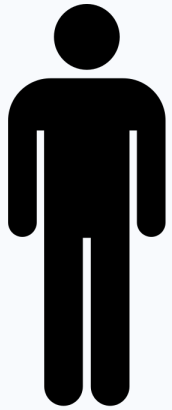
- GRAM + ROD-SHAPE BACTERIA → LISTERIOSIS
- OPTIONAL ANAEROBIO, NO CAPSULE, ASPORIGEN
- FLAGELLA
- + for CATALASE TEST, - for OXIDASE TEST
- GROW ON RICH SOIL
- FOUND in SOIL, FODDERS and WATERS
- GROW in CULTURE MEDIUM
  (BLOOD AGAR, OXFORD-LISTAR AGAR, OTTAVIANI AGAR)





CARRIER → CATTLE, SHEEP, GOATS

HUMAN INFECTION DERIVE BY CONTAMINATED FOODS FROM ANIMAL ORIGIN →



RESERVOIR OF INFECTION → RATS, TICKS, LIVESTOCK

LISTERIA TAXONOMY

Genus Listeria

Family Listeriaceae

Species:

- L. gray
- L. innocua
- L. ivanovii
- L. marthii
- L. monocytogenes
- L. murray
- L. recourtiae
- L. seligeri
- L. welshimeri

SEVERITY OF DISEASE

HIGH MORTALITY RATE (20-30%)

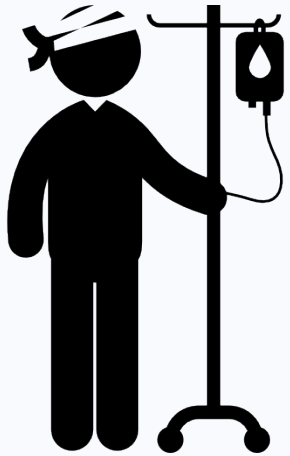
ECONOMIC AND SOCIAL IMPACT

RARE DISEASE 0.1-10 CASES / 1 MILLION / YEAR

LOW RATE OF CASES OF LISTERIOSIS
HIGH RATE OF MORTALITY FOR THIS DISEASE

SERIOUS PROBLEM OF PUBLIC HEALTH

WHO HAS A HIGHER RISK TO GETTING LISTERIA BY FOOD



- IMMUNOCOMPROMISED PERSON (HIV / chronic condition like CIRRHOSIS)

PREGNANT WOMEN
FETUS / NEWBORN

ELDERLY

LISTERIA TRANSMITTED BY FOOD

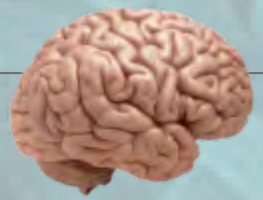
- FLU-LIKE SYMPTOMS
- PURULENT MENINGITIS
- MENINGOENCEPHALITIS
- SEPTICEMIA
- SERIOUS RIPERCUSSION FOR PREGNANT & FETUS

Contaminated food

Gut

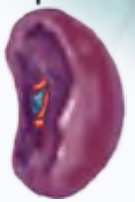
Lymph nodes

Liver



Brain

Blood stream



Spleen

Foetus

Placenta

WHY LISTERIA IS IMPORTANT IN RTE FOODS?

- ✓GOOD ADAPTABILITY: GROW AT LOW TEMPERATURE (+2/+4 C °)
- ✓RESISTANT TO CHEMICAL-PHYSICAL AGENT
- ✓LOW pH up to 5.0
- ✓[NaCl] up to 10%

IT HAS BEEN ISOLATED FROM FOODS OF ANIMAL ORIGIN WITH A LONG SHELF-LIFE

CONTAMINATION CAN ALSO COME FROM THE:
INADEQUATE APPLICATION OF HYGIENE and WORK PRACTICES
OBSOLETE PROCESSING ENVIRONMENTS

VS

THE OUTBREAKS and SPORADIC CASES of LISTERIOSIS are PREDOMINANTLY ASSOCIATED with the RTE FOODS (READY-TO-EAT)

The SEVERITY of the DISEASE and the FREQUENT INVOLVEMENT of INDUSTRIALLY TREATED FOODS, especially during OUTBREAKS, mean that the SOCIAL and ECONOMIC IMPACT of LISTERIOSIS is among the HIGHEST of FOODBORNE ILLNESSES.

Its ability to quickly produce biofilm allows it to survive for a long time, even up to 10 years, on surfaces of food production establishments, such as fishery products, heat-treated meat products and RTE cheese, of particular concern.

SO, RTE FOODS ARE DANGEROUS?

EATING CONTAMINATED FOOD WITH HIGH NUMBERS OF
Listeria monocytogenes
IS THE MAIN ROUTE OF INFECTION.
FOODBORNE LISTERIOSIS IS ONE OF THE MOST SERIOUS AND SEVERE FOODBORNE DISEASE.

2. HAZARD CHARACTERIZATION

1. INVASIVE LISTERIOSIS: infection to intestinal tissue, then spread to other organs with high mortality rate and symptomatic sequelae. Is characterized by a relative frequency of sporadic cases and by the occasional indication of actual outbreaks 95% of these sporadic cases are of food origin.
2. NON-INVASIVE LISTERIOSIS: observed in some outbreaks showing a gastroenteritis symptoms after a short incubation period.

Because the incidence rates and factors that govern the onset of the non-invasive form are currently still unknown, in the Quantitative Risk Assessment (QRA) study for L.m. in different types of food products ready for consumption, realized by FDA and USDA

(US Food and Drug Administration / US Food Safety and Inspection Agency),

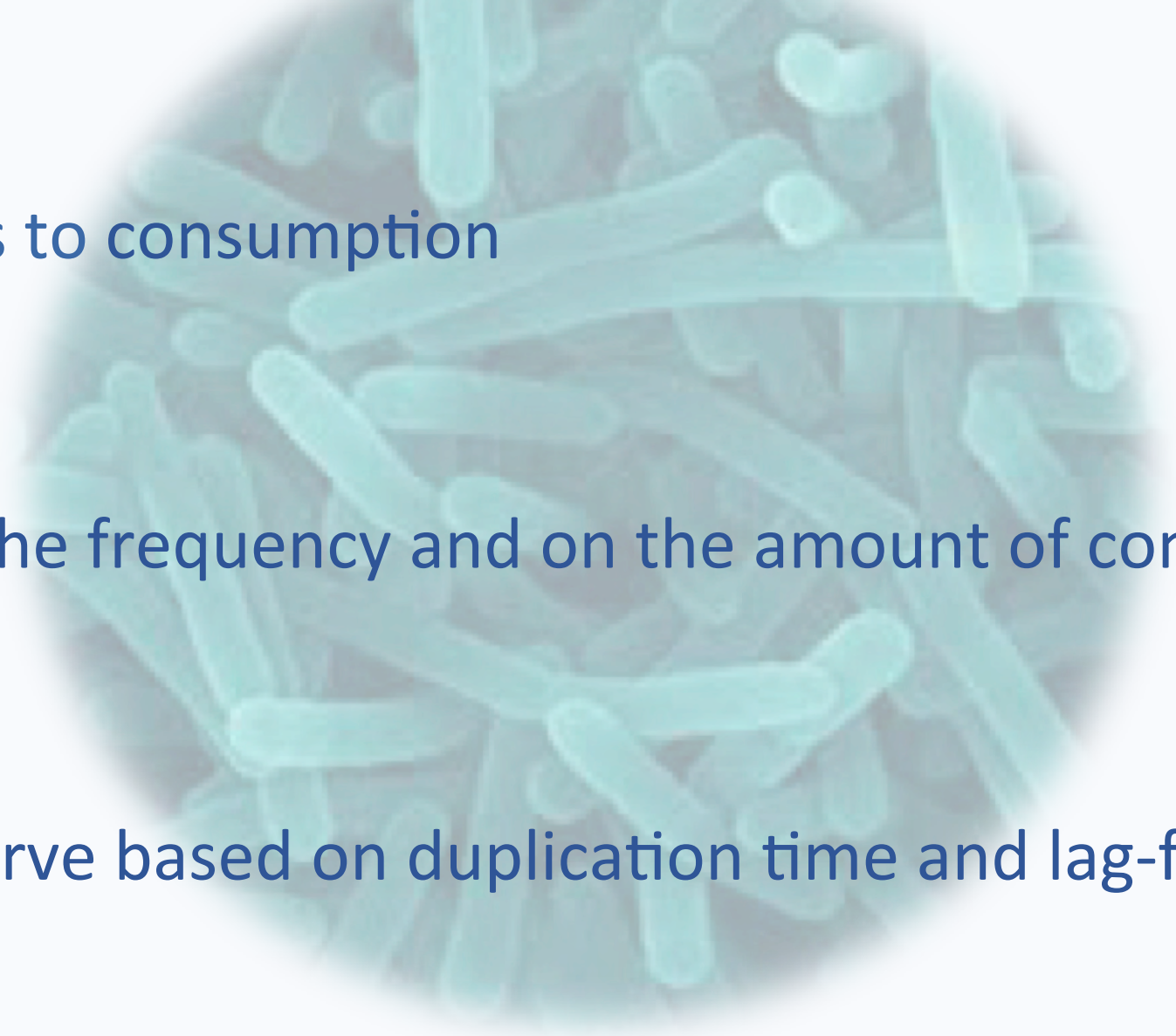
only the invasive form of listeriosis is considered.

EXPOSURE ASSESSMENT

Characteristics of RTE foods:

- Good substrate for the growth of *L.m.*
- Long shelf-life at refrigeration temperature
- Consumed without any healing treatment

EXPOSURE ASSESSMENT



- From sales to consumption
- Focus on the frequency and on the amount of consumption
- Growth curve based on duplication time and lag-fase time

EC 2073/2005 REG

- Microbiological criteria: -foodsafety criteria
  -criteria about the hygiene of the process
- RTE foods categories that must be respect this criteria
- Acceptability criteria and limits for RTE foods

EC 2073/2005 REG

RTE foods:

- Used for newborns or to special medical purposes
- Those that are a good substrate for the growth of listeria, different from those destinated for newborns or for special medical purpose
- RTE foods that aren't a good substrate for listeria growth

Acceptability limits for:

-Good substrate: shelf-life >5 d
pH>4,4
Aw > 0,92

-Bad substrate: shelf life < 5d
pH < 4,4
Aw < 0,92

Food products	REG CE 2073/05	Limits	Preventive or sanctionative action
Allow the growth -shelf-life >5d -Ph > 4,4 -Aw > 0,92	Yes	≤ 100 ufc/g	If *L.m.* ≤ 100 ufc/g: no action If *L.m.* > 100 ufc/g: withdraw and criminal offences
Allow the growth -shelf-file <5d -Ph > 4,4 -Aw > 0,92	Yes	Abscence/25g	If *L.m.* is absent: no action If *L.m.* ≤ 100 ufc/g: Withdraw If *L.m.* > 100 ufc/g: withdraw and criminal offences
Don't allow the growth -Shelf-life < 5d -Ph < 4,4 Aw < 0,92	Yes	≤ 100 ufc/g	If *L.m.* ≤ 100 ufc/g: No action If *L.m.* > 100 ufc/g: Wihdraw and criminal offences

THE MOST COMMON RTE FOOD AT RISK

	EU	ITALY
Dairy products Low level of contamination; good substrate Raw milk, soft cheese, pasteurized milk	0,47%(0.6%)	1%
Fermented meat-based products Often contaminated; no healing treatments; Do not allow the growth	2,07%(0,43%)	2,33%
Cold smoked fish Frequently contaminated; no healing treatments; Allow the growth	10,4% & 10,3% (1,7%)	20,3%(3,3%)

EXPOSURE ASSESSMENT

- The values times and the values temperature of conservation are taken and elaborated togheter through statistical methods
- Combining them with time of duplication and lag-fase time, is possible to do an estimation about the amount of *L.m.* in the food between the sales and consumption.

RISK CHARACTERIZATION

It aims to calculate the probability to contract listeriosis

The results are described with 2 risk estimates:

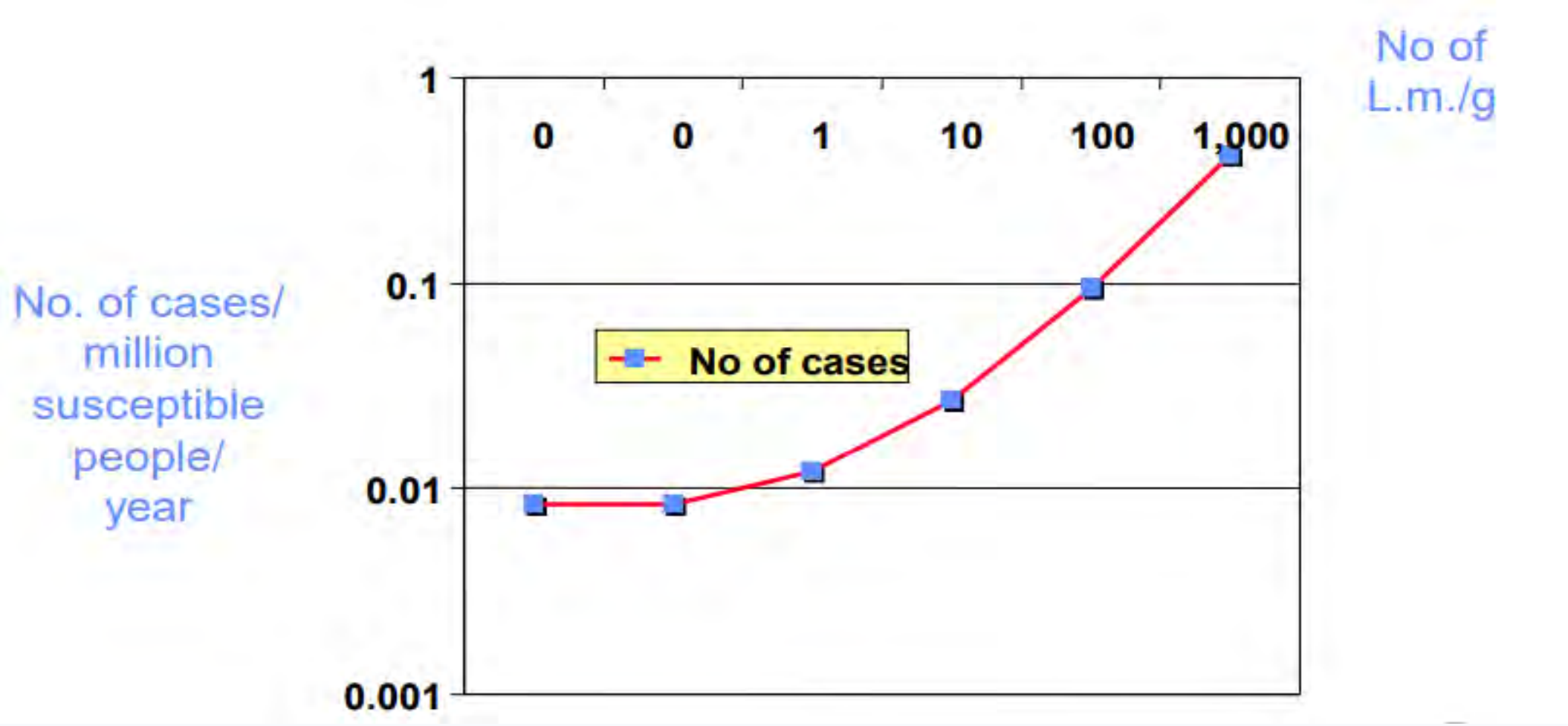
- for the consumer per one serving per year for different groups of people
- risks per million servings for healty and susceptible population

The mean risk estimates of the number of illness per 10 million people per year and the risk per serving for RTE foods

FOOD	CASES OF LISTERIOSIS PER 10 MILLION PEOPLE PER YEAR	CASES OF LISTERIOSIS PER 1 MILLION SERVINGS
Dairy products	9,1	0,005
Cold smoked Fish	0,46	0,021
Fermented meat	0,00066	0,0000025

To do an accurate *L.m.* risk characterization is important to evaluate 3 crucial points:

- The estimation to contract serious listeriosis eating foods containing amounts of *L.m.* that range from 0 to 1000 cfu in 25g



The risk for the most susceptible groups of people

Condition	Relative susceptibility	Calculate "r-value"
Transplant	2 584	1.4×10^{-10}
Aids	865	4.6×10^{-11}
Cancer – pulmonary	229	1.2×10^{-11}
Diabetes	25	1.3×10^{-12}
> 65 years old	7.5	4.0×10^{-13}
< 65 years old, healthy	1	5.4×10^{-14}

To understand if there are any differences between the food at risk and the others

- The combination of optimal conditions for the growth of *L.m.* influences the risk of contracting listeriosis

- The extent is dependent on the characteristics of the food and the condition and duration of the storage

CONCLUSIONS

- The risk to contract listeriosis eating contamined food is influenced by food matrix, bacteria virulence and consumer predisposition
- The risk to contract foodborne listeriosis is influenced by the food processing
- The risk to contract foodborne listeriosis is the same between EU and Italy but the processing can influence the contamination levels
- Most of the cases of listeriosis is linked with foods that don't respect the laws, either when the limit is 100 ufc/g, either when there is no tollerance.
- The control measures to reduce the frequency of contamination would include food processing methods that don't allow the growth of *L.m.*, decreasing the incidence rate of listeriosis

Thanks for the Attention